African Swine Fever

Disease Monograph Series – 08

Virus | Asfarviridae | Pigs

IDRC | Bartay

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Acronyms

ASF	African swine fever
ASForce	Targeted research effort on African swine fever
ASFV	African swine fever virus
ASEAN	Association of South East Asian Nations
AU-IBAR	African Union InterAfrican Bureau For Animal Resources
AU	African Union
AusAID	Australian Agency for International Development
BecA	Biosciences Eastern and Central Africa
CSIRO	Commonwealth Scientific and Industrial Research Organization
CVR	Central variable region
DISC	Defective infectious single cycle
dpi	Days post injection
DRC	Democratic Republic of Congo
ELISA	Enzyme-linked immunosorbent assay
EURL-ASF	European Union Reference Laboratory for African swine fever
FAO	Food and Agriculture Organization of the United Nations
FAT	Fluorescent antibody test
GARA	Global African Swine Fever Research Alliance

IL	interleukins
ILRI	International Livestock Research Institute
kb	Kilo base pair
MGF	Multigene family (e.g. 360 and 530 genes)
nm	nanometers
OIE	World Organization for Animal Health
PCR	Polymerase chain reaction
RT-PCR	Reverse transcription polymerase chain reaction
SAARC	South Asia Association for Regional Cooperation
TADs	Transboundary animal diseases
TNF	Tissue necrosis factor
USDA	United States Department of Agriculture
VI	Virus Isolation
VN	Virus Neutralization
WAHID	Interface for the World Animal Health Information System
WAHIS	World Animal Health Information System

Executive Summary

ASFV is a large, enveloped, double-stranded DNA virus that replicates primarily in cells of the mononuclear phagocytic system. It is currently classified as the only member of a family called ASF–like viruses (Asfarviridae) [1]. ASFV is the only known DNA arbovirus [1][4]. There are currently 22 known genotypes of ASFV [4][6]. The viral genome is differentiated using the VP72 (p72) gene, the p54-gene and the B602L-gene. ASFV is hardy and is able to survive for months or years in the environment, body fluids and in meat products. ASFV is able to evade host defenses since it does not induce neutralizing antibodies in the host pig and it undergoes constant internal reassortment of the genome [4]. Differences in the serological responses of pigs have been related to the specific immunological characteristics of the local breeds of African pigs [10].

The main strategy used by the virus to evade host defenses is to modulate the signaling pathway of infected macrophages in order to interfere with the expression of certain genes including those playing a role in the innate and acquired immunity [4]

ASF occurs in four clinical forms in pigs including peracute, acute, subacute or chronic forms. The incubation period is between 5 and 21 days in susceptible pigs having direct contact with infected pigs, but it can be less than 5 days after exposure to ticks. Acute disease typically appears in 3 to 7 days [20]. The OIE advises that since "*ASF cannot be differentiated from classical swine fever* [3] *by either clinical or post-mortem examination…laboratory tests are essential to distinguish between these diseases"* [3]. Ornithodoros ticks and wild pig species act as reservoirs and biological vectors of ASFV and wild pigs remain asymptomatically infected. Infected pigs that survive infection can act as carriers for months.

ASF was first described in Kenya in 1921 [11]. Since then, the virus has spread from wild African suidae (phacochoerus aethiopicus) to European domestic pigs brought to the African continent, causing 100% mortality. For decades it was confined to sub-Saharan Africa**,** until 1957 when it was detected in Lisbon, Portugal. ASF remains enzootic in most countries of Sub-Saharan Africa including Madagascar. In 2007, it was introduced the Caucasus (Georgia, Azerbaijan, and Armenia) and later to Russia and the Ukraine [6]. There are four main transmission cycles and distinct regional patterns of ASFV in Africa including: 1) sylvatic cycle; 2) tick-pig cycle; 3) domestic pig cycle; and 4) sylvatic to domestic pig cycle. The basic reproductive rate (R_0) for transmission is very high (3.24 in one case). Risk factors for transmission include moving infected pigs, human movement by veterinary and paraveterinarians, feeding food waste of infected pigs and soft Ornithodoros ticks.

The molecular epidemiology of ASFV in Africa provides evidence for the evolution of the virus over time as well as the epidemiological relationships among various disease outbreaks. There is a difference in the epidemiology and great diversity between the two distinct regions of Africa, based on molecular epidemiology [4]. In the Eastern and Southern parts of Africa, from Uganda and Kenya to South Africa, there are high levels of genetic variation with 22 genotypes described thus far, including 13 and 14 genotypes in Eastern and Southern Africa, respectively. This high genetic diversity is related to the occurrence of a sylvatic cycle in most of these countries.

The Western and Central parts of Africa have one unique genotype suggesting the absence of a genetic diversity driven by a sylvatic cycle [4].

Reporting of ASFV incidence to the OIE peaked in 2002 and 2013 [26]. Rwanda, Malawi and Madagascar in East and Central Africa accounting for over half of the total number of outbreaks between 2000 and 2015. The greatest impact of ASFV has been on the poorer pig producers who lack basic biosecurity and financial resources to resume production in the absence of compensation schemes [13]. Implications of ASFV for food security in Africa are significant and ASFV remains an imposing and enormous risk to animal health and food security globally.

The key challenges to developing a safe and effective vaccine for ASFV is due to the lack of understanding of the agent-host relationship including the following factors:

- Mechanism of evading innate and acquired immune systems i.e. macrophages and monocytes and acquired immune systems including both humoral and cell-associated aspects;
- The pig host does not develop neutralizing antibodies to ASFV;
- Phenotypic differences among known genotypes;
- Antigenic significance of approximately 150-165 genes of ASFV which can be used to target the development of safe and effective vaccines;
- Dynamics of going internal re-assortment and presence of multigene families (MGF) which impede the ability to develop effective and safe vaccines;
- Systematic coordination of basic research among international experts;
- Mechanism for technology transfer from researchers to vaccine companies for commercial vaccine production;
- Unique epidemiological drivers of ASFV of each area.

The key gaps and solutions that need to be addressed prior to developing a potential vaccine:

<u>Short term</u>

- Facilitating coordination among international researchers to advance knowledge of the basic science of ASFV through a systematic approach for antigenic and immune related research;
- Building local capacity for rapid detection including the development of a rapid pen side test for ASFV to support rapid diagnosis and permits differentiating infected from vaccinated animals (DIVA);
- Conducting epidemiological and socioeconomic studies at the country and transboundary levels to understand the drivers and impacts of ASFV, including introduction, transmission and endemicity;
- Leveraging efforts of research institutions through partnerships with the vaccine industry from the initial stages with potential to develop commercial products.

Medium term

- Studies of infection dynamics based on direct challenge and indirect contact from infected animals;
- Develop vaccine discovery models based on evidence from antigenic and immunological studies;
- Promoting technology transfer to private vaccine companies to support a multivalent delivery platform.

Long term

Developing vaccines and vaccine delivery systems appropriate for each country and region based on molecular, epidemiological and socioeconomic parameters.

Clinical disease overview

Etiology

The ASFV is hardy and can survive for long periods of time under unfavourable conditions. The OIE provides the following environmental information on resistance to physical and chemical inactivation of ASFV [5].

Virus structure

ASFV is a large, enveloped, double-stranded DNA virus that replicates primarily in cells of the mononuclear phagocytic system. It is currently classified as the only member of a family called ASF–like viruses (Asfarviridae) [1]. ASFV is closely related to the family Poxviridae sharing the following traits: 1) viruses from both families replicate mainly in the cytoplasm of the infected cells; and 2) their genomes have terminal cross-links and inverted terminal repeats [2]. At least 28 structural proteins have been identified in intracellular inclusion bodies. More than a hundred infectious proteins have been identified in infected porcine macrophages and at least 50 of them react with sera from infected or recovered pigs [3][4]. The virions contain the transcriptional machinery for the synthesis, capping and polyadenylation of early RNA. The inner envelope protein p54 is required for membrane recruitment to initiate the replication process [5]. Standard nomenclature for ASFV isolates includes city or country of isolation and last two digits of year of isolation (e.g. Lisbon '60, DR '78). ASFV is the only known DNA arbovirus [4][6].

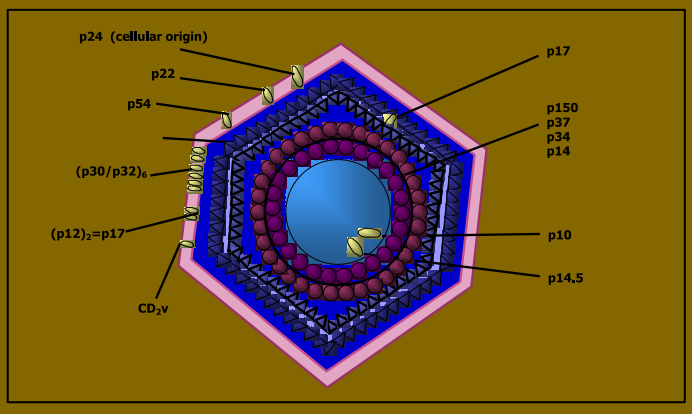

Figure 1: ASFV structure [4]

- Temperature: Highly resistant to low temperatures. Heat inactivated by 56°C/70 minutes; 60°C/20 minutes.
- pH: Inactivated by pH <3.9 or >11.5 in serum-free medium. Serum increases the resistance of the virus, e.g. at pH 13.4 – resistance lasts up to 21 hours without serum, and 7 days with serum.
- Chemicals/Disinfectants: Susceptible to ether and chloroform. Inactivated by 8/1000 sodium hydroxide (30minutes), hypochlorites – 2.3% chlorine (30 minutes), 3/1000 formalin (30 minutes), 3% ortho-phenylphenol (30 minutes) and iodine compounds.
- Survival: ASFV remains viable for long periods in blood, faeces and tissues; especially infected, uncooked or undercooked pork products. Can multiply in vectors (Ornithodoros sp.).

The molecular composition of ASFV is characterized by sequencing the C- terminal end of VP72 gene, which permits the differentiation of 22 distinct genotypes [4]. The full genome sequence of the *p54*-gene has been confirmed as a valuable additional genotyping method for molecular epidemiological studies. Enhanced discrimination is obtained by analysis of the CVR within the B602L-gene, described as the most variable locus to identify virus subgroups within several of the 22 genotypes. Figure 2 illustrates the phylogenetic relationships of 67 European, American, and West and East ASFV isolates. Analysis of these 67 isolates reveals that West African and European ASFV isolates are classified within Genotype I according to partial sequencing of p72 and these isolates were further classified into four major sub-types on the basis of their p54 sequences [7].

In summary, the viral genomic differentiation is possible using the following methods:

- The VP72 (p72) gene;
- The p54-gene sequencing confirms the separation of viruses into three additional clusters (V, X, and XX) that were homogeneous using p72 [7][8];
- The B602L variable region differentiates multiple sub-groups region. Using isolates obtained over a 40-year period following passage in either pig macrophages or *O. erraticus* ticks, isolates, which were previously grouped together, could be further differentiated [9].

ASFV remains one of the most challenging animal viruses because the virus does not induce neutralizing antibodies and its genome undergoes a highly variable and constant internal reassortment [4]. For these and other reasons, ASFV is endemically established in Africa. Serological tests are recommended where the disease is endemic or where a primary outbreak is caused by a strain of low virulence or avirulent [4]. One comparative serological study concluded that the challenge of serological diagnosis is not attributable to antigenic polymorphism, but rather may be related to the specific immunological characteristics of the local breeds of African pigs [10].

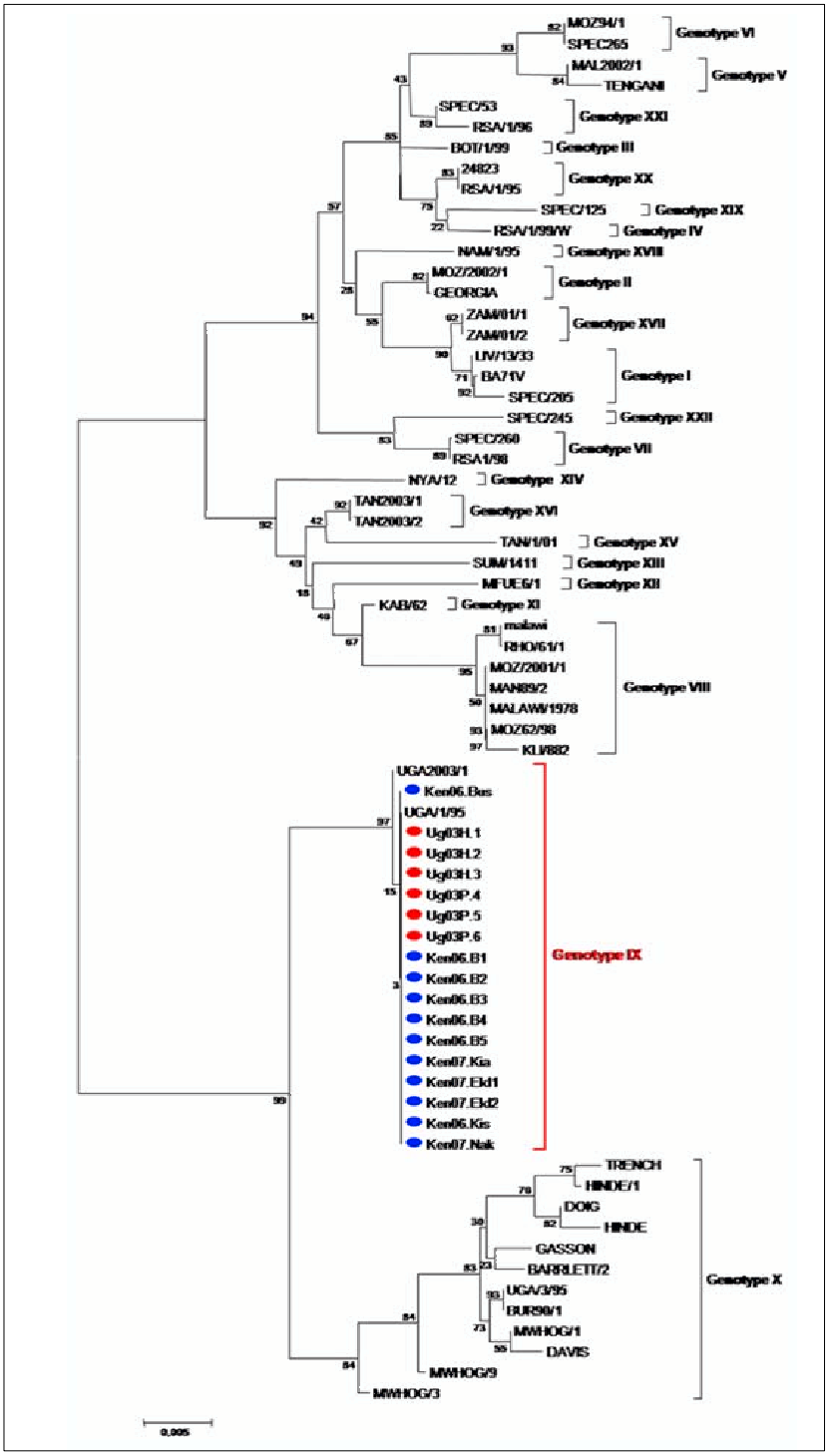

Figure 2: Genetic relationships of 67 European, American, and West and East ASFV isolates

Epidemiology

Table 1 summarizes key epidemiological features of ASFV to be further explained in greater detail under agent, host and environmental headings below [6].

Table 1: Key epidemiological features of ASF

Reservoirs inapparently infected from Africa	• African wild swine (warthogs, (*Phacochoerus aethiopicus*)) Bush pigs (*Potamochoerus* sp.) • Giant forest hogs (*Hylochoerus meinertzhageni*)
Hosts demonstrating symptoms	• Domestic pigs (*Sus domesticus*), • European wild boar • American wild pigs
Host and vector	• Ticks of the genus *Ornithodoros* are considered natural arthropod host
Transmission	• Direct transmission between sick and healthy animals • Indirect transmission by feeding garbage containing infected meat (ASFV can remain infectious for 3–6 months) • Biological vectors – soft ticks of the genus *Ornithodoros* • Fomites (premises, vehicles, implements, clothes) • Within tick vector: transstadial, transovarial, and sexual transmission
Virus sources	• Blood, tissues, secretions and excretions from sick and dead animals

Montgomery first described the disease in Kenya in 1921 [11]. The virus spread from wild African suidae (phacochoerus aethiopicus) to European domestic pigs more recently brought to the African continent, resulting in 100% mortality. For decades ASFV was confined to sub-Saharan Africa**,** until 1957 when it was detected in Lisbon, Portugal having spread from Angola, in the form of a peracute disease with high mortality. Figures 3, 4 and 5 depict the spatial distribution of ASFV during the time periods 1957-1967, 1997-2002 and 1998-2010 [11].

Figure 3: Spatial distribution of ASFV from 1957-1967

Figure 4: Spatial distribution of ASFV from 1997-2002

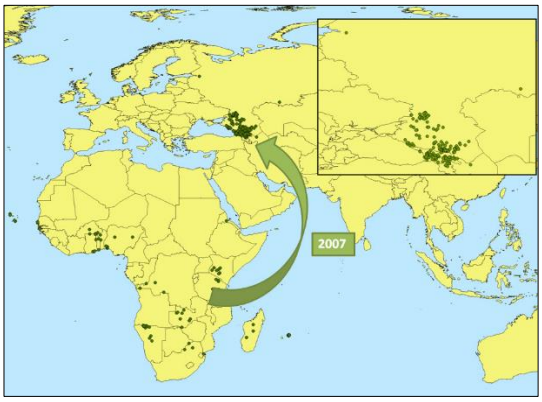

Figure 5: Spatial distribution of ASFV from 1997-2002

ASF remains endemic in most countries of Sub-Saharan Africa including Madagascar. In Europe, it has been reported and successfully eradicated from the Iberian Peninsula but continues to be found in Sardinia, Italy. In the 1970s, ASFV was present in the Caribbean (Haiti and the Dominican Republic) and one country in South America (Brazil) but was successfully eradicated. Most recently, it has appeared in the Caucasus (Georgia, Azerbaijan, and Armenia) and Russia [6].

The four main transmission cycles and distinct regional patterns of ASFV with reference to Africa are summarized in Table 2 and Table 3 below [12][13][14].

1. Sylvatic cycle

The sylvatic cycle has been documented in southern and Eastern Africa, where it involves both warthogs and ticks of the Ornithodorous moubata group. Young suckling warthogs are infected in burrows infested with soft ticks. A short period of viraemia follows, enabling transmission of ASFV to naïve ticks during blood meals. Warthogs remain asymptomatically infected for life, but due to the absence of horizontal and vertical transmission among warthogs, further transmission is dependent on O. moubata ticks. However, the presence of both warthogs and ticks in a region does not necessarily imply the existence of a sylvatic cycle. So far, a sylvatic cycle has not been documented in West Africa since warthogs or soft ticks are rarely infected. Studies in Eastern and Southern Africa showed that infection rates of free- living warthogs were rarely below 80 per cent in areas where the tick vector was present.

2. Tick-pig cycle

Ornithodorous ticks commonly feed on domestic pigs and can be involved in the transmission and long-term maintenance of ASFV. In Madagascar, ASFV was isolated from ticks found on a farm where no pig had been introduced for at least four years. However, the risk of ASFV infection decreases when tick populations become extinct following the absence of hosts over an extended period of time. Experimental transmission to pigs was demonstrated from ticks that tested positive within 380 days following an outbreak.

3. Domestic pig cycle

Once introduced into domestic pig populations, ASFV is transmitted through direct contact and by fomites. As previously noted, ASFV persists in blood and tissues for months so many animals can become infected at one location. Transmission through direct contact between domestic pigs can occur for up to 30 days after infection, or for eight weeks in the case of contact with blood as occurs during fighting or mating. Both pig trading and pig movement may be accelerated during an outbreak and the lack of biosecurity practices all contribute to the local spread of ASF in endemic areas.

4. Transmission from sylvatic to domestic cycles

Possible mechanisms for transmission of ASFV to occur in this way include the following:

- Soft tick vectors are the most likely means of transmission from African wild pigs to domestic pigs;

- Interbreeding between wild and domestic pigs may occur;
- Feeding infected wild pig meat to domestic pigs may also occur.

Factors found to increase the risk of outbreaks in domestic pigs are summarized in Table 2.

Table 2: Risk factors associated with occurrence of ASFV with references

Risk Factor	Reference
Pigs are unconfined and free-ranging	Allaway et al., 1995; Edelsten and Chinombo, 1995; 248 Mannelli et al., 1997
Previous occurrence of the disease on the farm	Randriamparany et al., 2005
Presence of an infected pig farm in the neighbourhood or of an abattoir in the community and	Fasina et al., 2012
Visits by paraveterinarians and veterinarians	Fasina et al., 2012
Density of the road network, of water bodies and of the domestic swine population	Gulenkin et al., 2011
Spread model shows the movement of infected animals to be the most important factor for ASF transmission	Olugasa and Ijagbone, 2007
Emergency sale of asymptomatic pigs	Babalobi et al., 2007; Costard et al., 2009b; Fasina et al., 2010; Randriamparany et al., 2005 Costard et al., 2012b
Feeding of bush pig meat to domestic pigs.	Sánchez-Vizcaíno et al., 2009
Rapid transmission and spread. High basic reproductive rate (R_0) for ASF virus in smallholder, free-range pig production systems	Barongo et al., 2015

Barongo et al. estimated the basic reproductive rate (R_0) for ASF virus in smallholder, free-range pig production system in Gulu, Uganda as 3.24 outbreaks generated per infected source [15].

Agent Factors

The stability of ASFV is described below for different isolates and matrices [4].

1. Pig feces:
    - 60 to 100 days.
2. Laboratory medium or serum:
    - 6 years at 5°C in the dark;
    - 18 months in serum at room temperature;
    - up to one month at 37°C;
    - 3.5 hours at 56°C (note that serum is normally safely sterilized after 30 minutes at 60°C).
3. Pork products:
    - 140 days in Iberian and white Serrano hams;
    - 399 days in Parma ham;
    - 112 days in Iberian pork loins.

Like other large DNA viruses, ASFV has developed a large range of defense mechanisms to escape from the immune host responses. In vivo, the virus replicates in macrophages, which are part of the innate immune system responsible for: 1) capturing and presentation of antigens to lymphocytes (acquired immune mechanism) of cell mediated immune systems; 2) engulfing and ingesting microbes independently or antibody coated (opsonized) pathogens and 3) secreting pro-inflammatory cytokines. ASF virus can persist in the natural hosts and in domestic pigs, which recover from infection, indicating that ASFV can evade host defense systems [4]. The main strategy used by the virus to evade host defenses is to modulate the signaling pathway of infected macrophages in order to interfere with the expression of certain genes including those playing a role in the innate and acquired immunity [4].

Using sera of infected animals it is possible to distinguish ASFV isolates based on the inhibition of the virus haemadsorbtion. Based on this phenomenon, pigs surviving infection or pigs infected with low virulent isolates generate antibodies that inhibit virus-specific haemadsorbtion have been divided into three groups, namely A, B, and C. However this categorization is not able to fully distinguish ASFV isolates [4].

Variability studies show that viral DNA is made up of a more preserved central area (B602L region of the ASFV genome) and two variable areas at the ends of the molecule, where diversity among isolates is greatest. As previously noted, different ASFV isolates have been classified in groups based on the relatively conserved central area [15].

The molecular epidemiology of ASFV in Africa provides evidence for the evolution of the virus over time as well as the epidemiological relationships among disease outbreaks.

Figure 6 presents the geographic distribution and phylogram of ASFV genotypes in Africa recently summarized by Costard et al, 2010 [13][15].

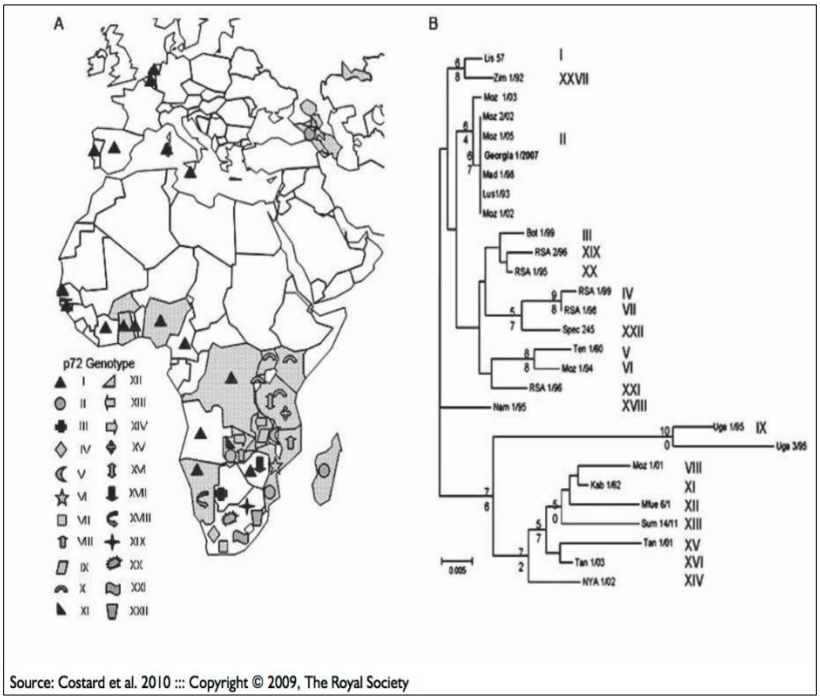

Figure 6: Geographic distribution and phylogram of ASF genotypes in Africa

The differences in genome size and enzymatic restriction profile among isolates show a high level of antigenic variability due to a change in the number of genes in a MGF, resulting in a large diversity between isolates through homologous gene recombination. A reduction in the number of MGF genes seems to be associated with lower virus virulence. Comparison of the ASFV genomes showed that 85% of the encoded proteins were identical between viruses, the more variable ones belonging to the MGF [4].

In Africa, attention is focused on the evolution of the circulating strains, from the molecular and the biological point of view as a means of explaining mechanisms of virus maintenance. [4].

On the African continent, ASFV has been endemic and circulating for a long period of time with minimal intervention. As a result, there is great diversity within two distinct regions of Africa based on molecular epidemiology [4]:

- The Western and Central parts of Africa, from Namibia to DCR and to Senegal, where the unique genotype I is circulating. Its high homogeneity does not reveal the exact origin of the virus exported from Eastern Africa to Western Africa and Europe or to explain the difference between outbreaks occurring as early as 1959 and those as recently as 2000 in West Africa. However, this homogeneity supports studies demonstrating the absence of a sylvatic cycle in West Africa in contrast to the Eastern and Southern African countries.
- Isolates from the Eastern and Southern parts of Africa, demonstrate high levels of genetic variations. A total of 22 genotypes are currently described, with 13 and 14 genotypes in Eastern and Southern Africa, respectively. This high genetic diversity is related to the presence of a sylvatic cycle, which plays a crucial part in the transmission of the disease. Zambia currently has seven (7) genotypes, South Africa has six (6), Mozambique has four (4), Malawi and Tanzania each with three (3), and Kenya and Uganda each with two (2). Some genotypes (VIII and XIX) are extremely homogeneous and seem to be associated to pig-restricted cycles or pig-domestic tick exchanges. Other genotypes (V, X, XI, XII, XIII, XIV) were either isolated from domestic pigs, wild ticks or warthogs in both sylvatic and domestic cycles. Some genotypes are more country specific (V, VI, IX, XI, XIII, XIV, XV and XVI) while others (I, II, V, VIII, X and XII) are not restricted by national boundaries.

Using *p72* and other molecular markers such as *9RL,* molecular genetics can be to distinguish viruses causing outbreaks that are geographically or temporally related. Several case studies are presented [4]:

- ASF outbreaks occurring in Uganda in 1995 caused by two different viruses are associated with the 1984 and 1990 outbreaks in Burundi.
- Outbreaks occurring in South Africa in 1995 and 1996 are believed to represent two unrelated epizootics including four genotypically unrelated viruses. These results contrast with the recovery of the same unique genotype from the 1987, 1992 and 1996 outbreaks in South Africa, indicating endemicity of this virus.
- ASF viruses recovered from the single outbreak focus in 1998 in Mozambique belonged to the two unrelated genotypes II and VIII. The same genotypes occurred in two different regions of Mozambique in 1994 (Bastos et al. 2004). Prolonged presence of both genotypes has been supported by the recovery of a genotype II virus from outbreaks in Nampula and Cabo Delgado provinces of Mozambique between 2001 and 2003, and a genotype VIII virus from an outbreak in Zambézia Province in 2001.
- The parallel identification of the genotype II in Madagascar indicates that Mozambique was the most likely source of infection for the 1998 introduction of ASFV into Madagascar.

Host Factors

ASF is a very complex viral disease that affects only wild and domestic porcine species [10]. Pathogenesis mechanisms of infection by ASFV of different virulence are not yet well understood and pig host immune responses to ASFV infection are complex. Antibodies *per se* do not appear to protect against infection and more recent studies emphasize innate cellular and cellular based immune mechanisms as relevant for animal survival. ASF can be transmitted directly from diseased or carrier pigs to healthy swine though direct or indirect contact or indirectly through fomites, ingestion of raw infected pork or pork products, or by biological vectors such as Ornithodoros ticks [4].

It is essential to consider the specific epidemiological host factors for each area as unique. For example, the maintenance of the virus in domestic pigs are more important than the association with warthogs as a cause of outbreaks of ASF in most parts of Mozambique [16]. The wild suids such as warthogs in Eastern Africa play a large role in eastern Africa, whereas this reservoir host does not play a significant role in western Africa [17]. Maintenance of ASFV in domestic pig populations is also specific in each area and for each outbreak. Interestingly, European wild boars are usually more resistant than domestic pigs to ASFV infection, although they present a similar pathological and epidemiological pattern [17].

Infected pigs shed the virus during the incubation period (15-21 days) and for up to 48 hours before the onset of clinical signs of disease. During the clinical stage of disease, higher levels of virus are present in blood, secretions and excretions. Pigs that recover may shed virus for up to a month after the disappearance of clinical signs [18].

The co-existence of different ASFV genotypes in warthog burrow-associated ticks and adult wild warthogs was demonstrated in one endemically infected area in East Africa. The data from this and earlier studies suggest transfer of viruses of at least two different p72 genotypes, from wild to domestic pigs in East Africa [19].

Environmental Factors

Understanding the ecology of the interface between natural and farm ecosystems, ASF hosts, carriers and vectors is critical to the epidemiology and risk factors for ASFV in different regions of the world. ASFV replicates in swine and in soft ticks of the genus Ornithodoros. However, there are different epidemiological cycles or scenarios depending on the specific circumstances in each geographical area regarding virus strain, host susceptibility, and biological vector presence and/or vector interaction with susceptible hosts. Pig production and management systems strongly influence the epidemiology of the disease as well as the prevention and control efforts required. The following examples are presented:

- Eastern Africa: ASFV is maintained mainly in a sylvatic cycle involving *O. moubata* vectors and warthogs, complicating control efforts;

- Western Africa: transmission between domestic pigs due to uncontrolled movement; Iberian Peninsula: *O. erraticus* was mainly associated to domestic rather than wild suids habitats; effectively control by replacing the old pigsties by modern structures;
- Sardinia, Italy and some African countries: the disease is maintained by free range/backyard production systems where recovered pigs act as carriers;
- Caucasus: contacts between diseased wild boar and free ranging pigs play an important role in the spread of ASF [4].

A more detailed tabular summary of evidence for ASFV occurrence in East and West African ecological systems is presented in Table 3 [12].

Table 3: Evidence of ASFV occurrence in East and West African environments [12]

Regional Evidence	Reference
East and South Africa	
Sylvatic cycles with sporadic emergence in domestic pigs have been described in these regions; in Kenya and South Africa, Genotypes I, X and XX isolates were recovered from warthogs and ticks found nearby to affected pig farms	Heuschele and Coggins, 1965b; Pini and 394 Hurter, 1975
In Malawi, the existence of both tick-pig and domestic cycles has been demonstrated; in the endemic central region of the country, ASF was shown to affect farms with soft ticks in areas without warthogs	Haresnape, 1984; Haresnape et al., 1985, 1987, 1988; Haresnape and Mamu, 1986; Haresnape and Wilkinson, 1989
In Zambia, the studies suggest the existence of a domestic cycle, with sylvatic cycles restricted to national parks and their adjacent areas.	Wilkinson et al. 1988 and Samui et al. 1996
In Mozambique, ASF is endemic in regions close to Malawi and Zambia but outbreaks are reported throughout the country. A sylvatic cycle may occur in some national parks and be the cause for important pig losses in the surrounding areas	Penrith et al., 2007
West Africa	
In West Africa, the domestic cycle is the only one involved in the persistence of ASFV	Sanchez-Vizcaino et al., 2009
In Senegal, epidemiological studies and molecular typing suggest that warthogs and *O. sonrai* ticks are unlikely to be involved in transmission of	Bastos 423 et al., 2003; Jori et al., 2007; Vial et al., 2007; Etter et al., 2011

the disease. High serological prevalence was found in farms where animals did not exhibit clinical signs	
Madagascar	
Recent studies suggest that ASF is restricted to the domestic pig population, with seasonal peaks of ASF and mostly unspecific symptoms reported by farmers. An abattoir survey conducted in 2006 found up to a quarter of slaughtered pigs infected with ASFV	Costard et al., 2009b
Both bush pigs and *O. moubata* ticks are present on the island, but no evidence of bush pig-ASFV or bush pig-tick was detected. Molecular epidemiology supports these findings, with all circulating viruses belonging to Genotype II	Roger et al., 2001; Jori et al., 2007; Ravaomanana et al., 2010, 2011; Bastos et al., 2003

Clinical Signs

ASF can be observed in pigs in four clinical forms: 1) peracute; 2) acute; 3) subacute; or 4) chronic disease. The incubation period is 5 to 21 days after direct contact with infected pigs, but it can be less than 5 days after exposure to ticks. Acute disease typically appears in 3 to 7 days [20]. Table 4 summarizes the clinical features of four clinical forms of ASFV based on experimental evidence in pigs [4][21]. Wild African pigs are very resistant to infection and when infected, they are asymptomatic.

Table 4: Clinical manifestations of ASFV in the pig host based on experimental challenge model [4][21]
http://sanidadanimal.info/cursos/asf/caps/cap6.html

Death (dpi)	Clinical Signs
Peracute, high virulence	
80-100% 7 dpi or less	• Sudden death
Acute, high virulence	
80-100%	• Fever 40-42 ^{O}C • Recumbent, anorexia, huddling, cyanosis of ears;

	• Final stages: rapid laboured breathing; serous or seromucous nasal secretions; nasal haemorrhaging, constipation and vomiting, and to a lesser extent, diarrhoea; melena; • Exanthemas (pinkish almost purple skin due to intense hyperaemia) or cyanotic foci, which appear as irregular purple-coloured marks on the skin of the extremities, ears, chest, abdomen and perineum; • Abortion in gestating females; • Death within 6-13 days, or as late as 20 days; survivors are carriers for life In domestic swine, the mortality rate often approaches 100%.
Subacute, moderate virulence	
30-70% mortality 7-20 dpi	• Clinical signs develop more slowly and the disease produces temporary thrombocytopenia resulting in death • Haematomas and necrotic areas, though these lesions • Irregular recurring fever for up one month, followed in most cases by recovery; • Duration of illness up to 30 - 45 days; • Anorexia and loss of condition; • Coughing and dyspnoea; • Exercise intolerance and death due to cardiac pathology; • Abortion in pregnant sows; • Mortality rate is lower but varies widely.
Chronic, mild virulence	
2-10% mortality	• Variety of clinical signs which are mainly the result of secondary bacterial complications; • Abortions; • Necrosis of the skin and buccal cavity, as well as arthritis, lameness; • Weight loss, growth retardation of growing pigs which have a long hairy coat, irregular peaks of temperature, respiratory signs • Lameness caused by arthritis that due to necrosis of cartilage; • Animals are vulnerable to secondary infections and pneumonia over 2-5 months • Low mortality (less than 30%)

Diagnosis

The diagnosis of ASFV utilizes VI and serological evidence of exposure, depending on the laboratory capacity available [3]. The following factors should be considered [3][21]:

- Early detection is critical since there is currently no effective vaccine;
- There are no neutralizing antibodies, there are no multiple serotypes, only genotypes;
- Viraemia begins during the incubation period and can be prolonged;
- Persistence of antibodies for months or years;
- Infection results in antigen-antibody complexes related to vascular endothelial damage.

The following tissues from filter organs of dead animals are to collected and preserved under cold chain conditions for diagnosis: lymph nodes; spleen; kidney; lung; as well as whole blood and serum [3][21].

Differential Diagnosis

Table 5 presents a summary for the main differential diagnoses of ASF [21].

The OIE advises that since "ASF cannot be differentiated from classical swine fever (hog cholera; Classical Swine Fever) by either clinical or post-mortem examination, and both diseases should be considered in the differential diagnosis of any acute febrile haemorrhagic syndrome of pigs. Bacterial septicaemias may also be confused with ASF and CSF. Laboratory tests are essential to distinguish between these diseases" [3].

Table 5: ASF differential diagnoses

Disease	Affected species	Signs		Lessions	
		Coinciding	Differential	Coinciding	Differential
Classical Swine Fever	Pigs	Fever, depression	Longer clinical course than ASF	Cutaneous and renal haemorrhaging, and in the lymphatic ganglia.	Ulcers in the caecum and colon, marginal infarct in the spleen (**Fig.39**), pale renal parenchyma, non-purulent meningoencephalitis. (**Fig. 40** and **41**)
Acute salmonellosis (S.cholerasuis)	Pigs	Swine Fever, abortions.	Yellowish liquid diahrroea, low morbidity and high mortality.	Cyanosis on the tips of the ears, tail, trotters and abdomen, haemorrhaging in the renal cortex, splenomegaly.	Focal hepatic necrosis, serous or necrotic enterocolitis.
Erysipelas (Swine Erysipelas)	Pigs	Swine fever.	Chronic forms of arthritis.	Splenomegaly, petechiae in the renal cortex, ganglionar hypertrophy with tumefaction and haemorrhaging. Romboid urticariform lesions on the skin.	Arthritis and vegetative endocarditis.
Dermatitis-nephropathy syndrome	Pigs	(See lesions)	Non-specific, slight hyperthermia, weakness	Purple-red marks on the skin of the hams, ears, abdomen and perineum.	Renal petechiae. Lesions caused by necrotising vasculitis. Pale kidneys despite petechiae.
Aujeszky´s disease.	Pigs, ruminants, rodents and carnivores.	Abortions (**Fig. 42**), cutaneous cyanosis in piglets. (**Fig.43**)	Nervous signs	Pneumonia.	Necrotic enteritis.

Pathology

Pig macrophages are the main targets for viral infection. Deeper characterization of viral interactions with these cells, and with the domestic pig as a natural host, using viral isolates characterized at genome level (naturally obtained or experimentally manipulated) is required in order to gain new insights to facilitate the manipulation of pig immune responses This will enable induction of protective immune responses and contribute to the development of effective vaccines [4].

The virus interaction with the macrophage and monocytic cells in the immune system produce characteristic haemadsorption before the infected cell is destroyed. Viral replication has also been observed in endothelial cells, hepatocytes, renal tubular epithelial cells and neutrophils. However, Infection has not been described in T or B lymphocytes [20]. The gross pathological lesions of ASFV are summarized in Table 6 [21].

Table 6: Characteristic pathological lesions of ASFV

Form	Characteristic Lesions
Peracute	• Severe pulmonary oedema, xanthema; • Hyperaemic splenomegaly; • Haemorrhage: lymphatic ganglia, kidneys, bladder, pharynx, larynx, endocardium, pericardium; • Hydropericardium.
Acute	
Subacute	• Haemorrhage and necrosis: lung, kidneys, gall bladder, pharynx, larynx, endocardium, pericardium, submandibular and retropharyngeal lymph nodes, mediastinal, inguinal and mesenteric ganglia, serous layer of the small and large intestine, fundic mucosa of the stomach and skeletal muscle; • Fibrinous pericarditis.
Chronic	• Haemorrhage: renal and gastro-hepatic lymph ganglia and kidneys.

Diagnostic Tests

In developing countries, identification of the agent is possible in a few reference laboratories so sample must always be sent for confirmation to these recognized laboratories. The OIE identifies the following diagnostic techniques when ASFV is suspected [3]:

1. Identification of the agent.

    a. Haemadsorption test: The haemadsorption (HAD) test (Malmquist & Hay, 1960) is based on the fact that pig erythrocytes will adhere to the surface of pig monocyte or macrophage cells infected with ASFV and that most virus isolates produce this phenomenon of haemadsorption. A positive result in the HAD test is definitive for ASF diagnosis. Alternatively, the Haemadsorption 'autorosette' test with peripheral blood leukocytes from infected pigs, when obtaining timely results is of the essence.

    b. Antigen detection by FAT: The FAT (Bool et al., 1969) can be used as an additional method to detect antigen in tissues of suspect pigs in the field or those inoculated at the laboratory. Positive FAT plus clinical signs and appropriate lesions can provide a presumptive diagnosis of ASF. It can also be used to detect ASFV antigen in leukocyte cultures in which no HAD is observed and can thus identify non-haemadsorbing strains of virus.

    c. Detection of virus genome by the PCR: PCR techniques have been developed, using primers from a highly conserved region of the genome, to detect and identify a wide range of isolates belonging to

all the known virus genotypes, including both non-haemadsorbing viruses and isolates of low virulence. Two validated PCR procedures are described:

i. PCR amplification by conventional PCR (Agüero et al., 2003)

ii. PCR Procedure: TaqMan® PCR protocol (King et al., 2003)

2. Serological tests

Antibodies persist in recovered pigs for long periods after infection, sometimes for life, and a number of tests are available for detecting these antibodies.

a. ELISA (the prescribed test for international trade): The ELISA (Pastor et al., 1990) is a direct test that can detect antibodies to ASFV in pigs that have been infected by viruses of low or moderate virulence.

b. Indirect FAT: This test (Pan et al., 1974) should be used as a confirmatory test for sera from areas that are free from ASF and are positive in the ELISA, and for sera from endemic areas that give an inconclusive result in the ELISA.

c. Immunoblotting test (Escribano et al., 1990; Pastor et al., 1989): This test should be used as an alternative to the IFA test to confirm equivocal results with individual sera. The immunoblotting test is very specific and enables easier and more objective interpretation of the results and a better recognition of weak-positive samples.

Zoonotic disease

ASFV is not considered to be a zoonosis. However, viral sequences related to the afarvirus family but highly divergent from ASFV have been reported in human serum and sewage [23]. Detection of these sequences suggests that greater genetic diversity than previously though may exist among asfarviruses and this raises the possibility that human infection by asfarviruses may exist.

Incidence and Prevalence in Selected Countries

Global

European countries ASFV outbreaks include: France (1964), Italy (1967, 1969, and 1993), Malta (1978), Belgium (1985), and Netherlands (1986), Portugal (1999. ASF remains endemic on the island of Sardinia, Italy since 1978 but it has been eradicated in the other countries.

Countries in the American continents affected by ASFV include: Cuba (1971, 1980), Brazil (1978), Dominican Republic (1978) and Haiti (1979). ASF was successfully eradicated in all countries.

In 2007, ASFV was reported on the European continent, in the Caucasus region. Outbreaks were detected near the port of Poti, Georgia, likely related to infected pork meat that was brought by international ships. Subsequently, ASF spread to the neighbouring countries of Armenia, Azerbaijan and the Russian Federation, causing huge economic loses.

Figure 7 presents the global distribution of ASFV in 2015 [24]. Figure 8 presents a regional map of distribution of ASFV events in Africa during 2011 [25].

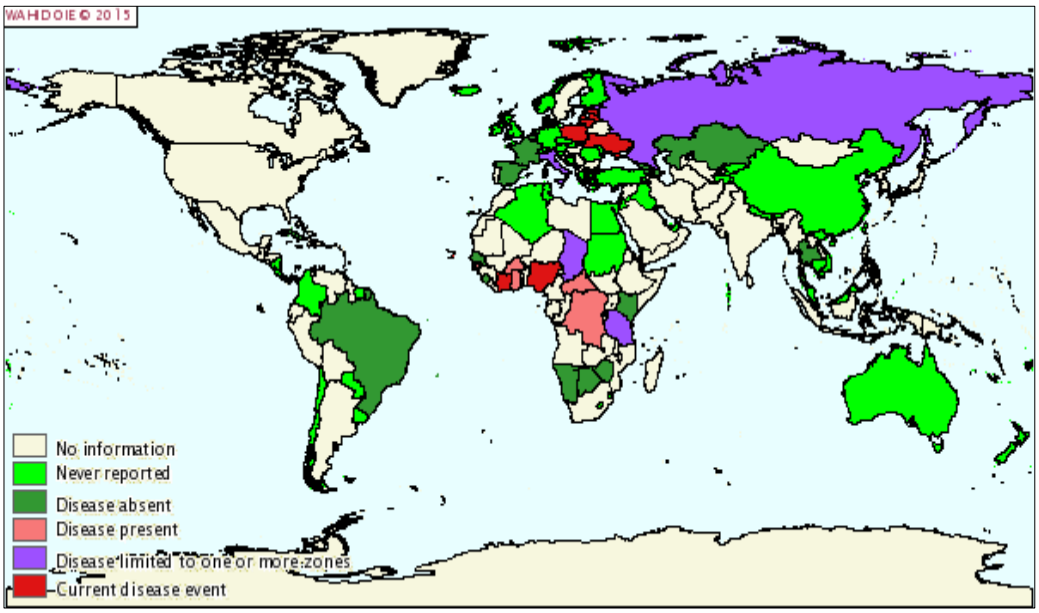

Figure 7: Global distribution of ASFV, 2015 [24]

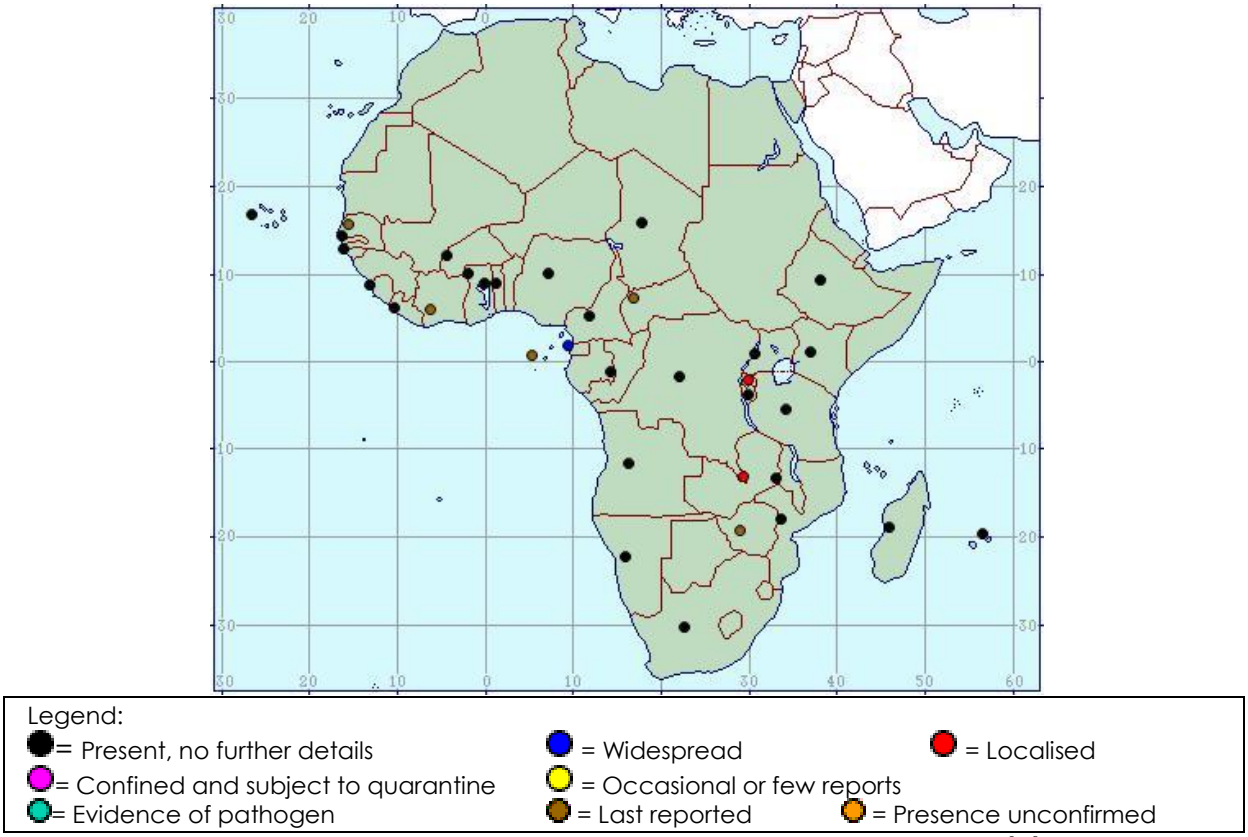

Figure 8: Regional map showing the distribution of ASFV events in Africa during 2011 [25]

Regional

Table 7: Incidence of ASF in 20 Selected Countries, 2000-2015 [26]

Region/Country	Reported Incidence ASFV [26] http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statusdetail# (Accessed 20 October 2015)															
	2000	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**
Sub Saharan Africa																
Burkina Faso	0	0	0	1	1	…+	0	2	5	34	33	27	52	37	7	…
Ethiopia	0	0	0	0	0	…	…	…	…	…	…	?	?	…	…	…
Ivory Coast	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	…
Kenya	0	3	0	0	0	0	0?	7	0	0	2	4	…+	0	0	1
Madagascar	33	…	72	41	22	17	7	…+	…+	…+	7	4	3	7	7	…
Malawi	23	41	16	9	26	24+	17	21	14+	6	35	42	19	33	…	…
Mali	0	0	0	0	0	0	0	…	…	…	…	…	…	…	…	…
Mozambique	8	8	5	6	15	16	13	11	12	7	11	12	6	9	4	…
Rwanda	…	…	51	99	…	…	64	29	26	6+	53	50+()	…+()	96	…	…
Senegal	0	4	3	3	1	2	2	6	0	1	0	0	0	0	0	0

South Africa	0	2	3	2	2	1	1	1	2	…+()	0	1+?	18	?+	3	…
Tanzania	0	15	1	16	4	1+?	0	0	4	…+	1…+	14	()+	19	7	4
Uganda	32	52	57	28	24	3	…+	3…+	2…+	…+	1…+	10	…	1	…+	…
Zambia	1	5	7	0	5	…	3+()	4	13	19	6	()+	1	23	12	…
South Asia																
Bangladesh	0	0	0	0	0	0	0	0	0	0	0	0	0	…	…	…
India	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	…
Nepal	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	…
Southeast Asia																
Indonesia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	…
Myanmar	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	…
Vietnam	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

WAHIS Codes 2005-2015

…	No information available for this disease
0	Disease absent

?	Disease suspected
? +	Infection/infestation
...+	Disease present but without quantitative data
+	Disease present with quantitative data but with an unknown number of outbreaks
+()	Disease limited to one or more zones

HandiStatus II Codes 2000-2004:

...	No information available

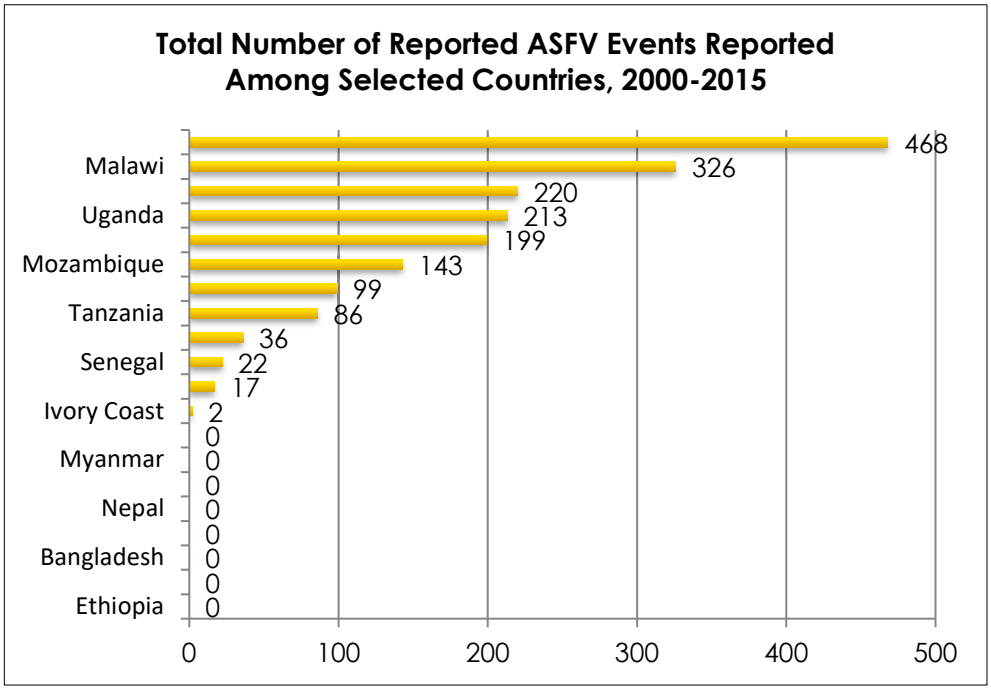

Figure 9: Total Number of Reported ASFV Events Reported by Selected Countries, 2000-2015

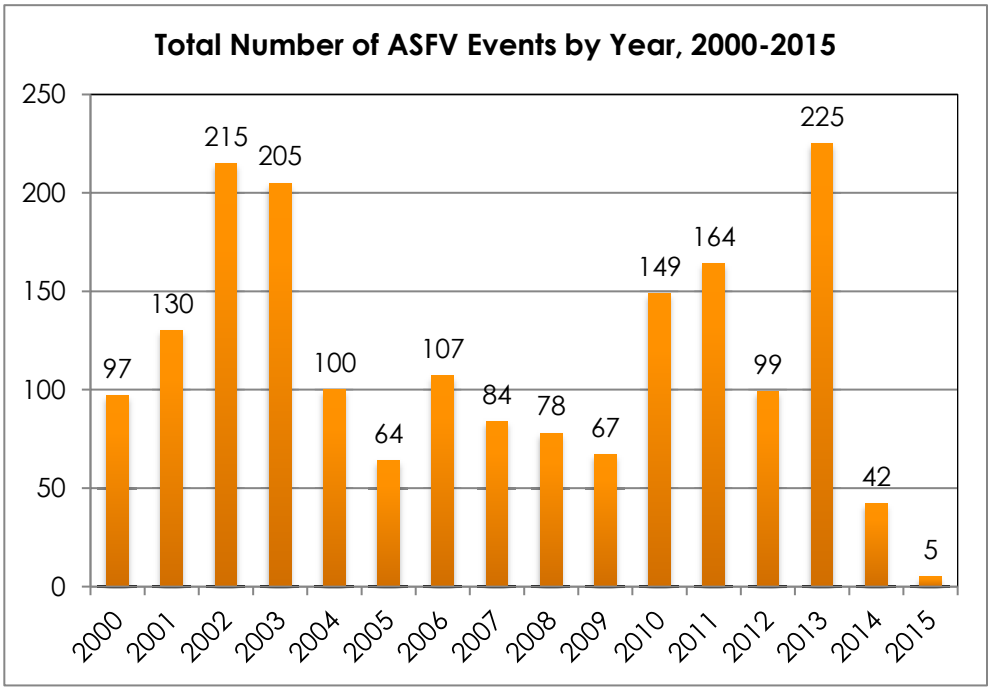

Figure 10: Total Number of Reported ASFV Events Reported year, 2000-2015

"During 2011, ASF outbreaks were reported to AU-IBAR by 22 countries with a total of 471 affected epidemiological units involving 144,950 cases, 135,712 deaths and a case fatality rate of 93.6%. Significantly, the DCR registered the highest number of outbreaks (84) accounting for about 17.8 % of the reported outbreaks and 79.4% of mortalities (AU-IBAR, 2011)" [25].

Table 8 presents a summary of the number of ASFV outbreaks from Africa reported in 2011 [25]. Figure 11 depicts the increasing historical temporal distribution of outbreaks in East, South, Central and West Africa until 2011 [27].

Table 8: Characteristic pathological lesions of ASFV

Country	Outbreaks	Cases	Deaths	Slaughtered	Destroyed
Benin	25	1426	815	536	52
Burkina Faso	26	1518	1134	0	0
Cameroon	4	146	89	0	NS
Central African Republic	17	993	742	0	0
Chad	7	189	126	59	54
Congo Brazzaville	1	2	2	0	0
DCR	84	105,614	105,614	9691	49
Ethiopia	7	28	19	0	NS
Gambia	5	198	198	0	0
Ghana	7	567	510	152	25
Kenya	6	57	53	0	NS
Liberia	1	12	4	8	0
Madagascar	19	540	540	NS	91
Malawi	36	19,755	18,956	114	19
Mozambique	16	591	380	0	316
Nigeria	1	1	70	0	0

Rwanda	60	677	600	2054	647
South Africa	1	NS	NS	NS	NS
Tanzania	7	2063	1334	NS	NS
Togo	80	2363	1151	235	40
Uganda	56	7788	3763	1584	99
Zambia	5	422	212	NS	NS
Total	471	144,950	135,712	14,433	1392

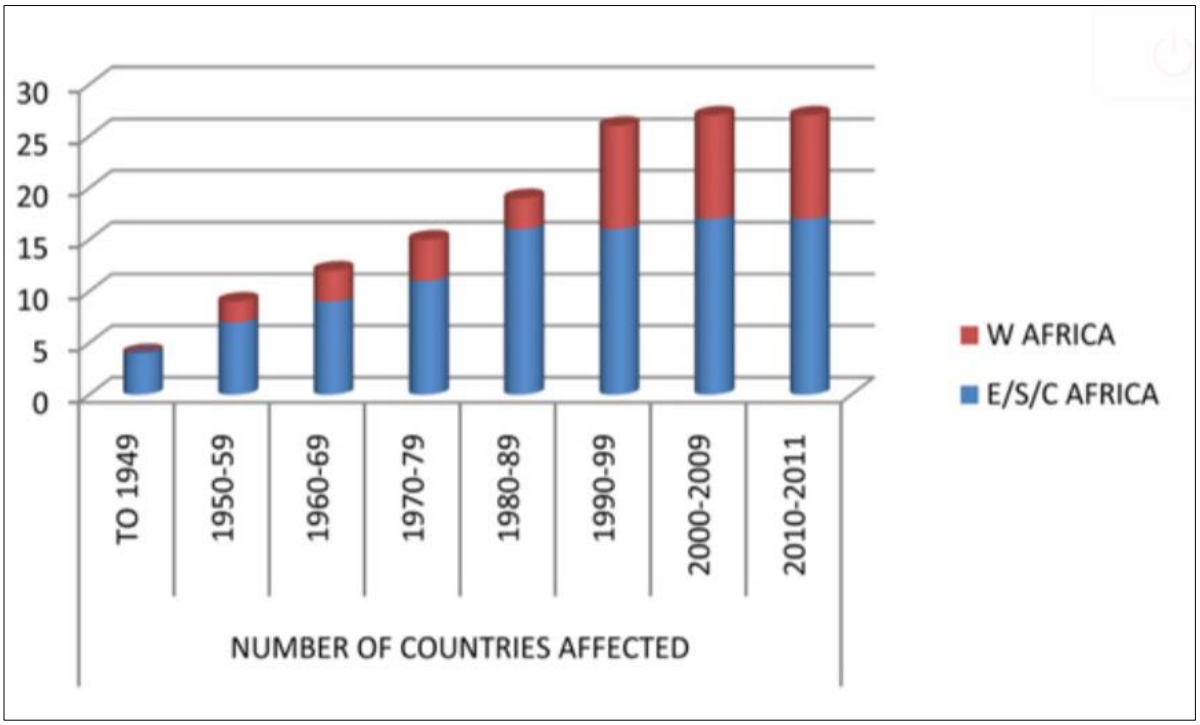

Figure 11: Total Number of Reported ASFV Events Reported year, 2000-2015

Table 9: Prevalence estimates of ASFV in selected African countries

Region/Country	Apparent Prevalence (CI)	Study Design	Time Period	Reference
Sub Saharan Africa				
Burkina Faso				
Ethiopia				
Ivory Coast				
Kenya	Analysis of blood and serum samples using a PCR assay demonstrated an average animal level positivity to ASFV of 28% in two independent samplings in South-western Kenya and 0% PCR positivity in Central Kenya.	Prospective cross-sectional study of seroprevalence and virus prevalence in two pig producing regions of Kenya.	2008-2009	Okoth et al, 2012
Madagascar	Prevalence estimates provided by the abattoir survey varied between areas: 1% in Ambatondrazaka, 10% in Marovoay and 24% in Arivonimamo areas of Madagascar	Prospective cross-section virus prevalence survey	2006	Costard et al., 2009
Malawi				
Mali				

Mozambique	The seroprevalence to ASFV was 12.6% on farms and 9.1% in pigs, while it reached 75% in warthogs. Approximately 33% of pigs and 78% of warthogs showed antibodies against salivary antigens of ticks.	Prospective cross sectional survey of domestic pigs and warthogs were sampled to determine the prevalence of antibodies against ASF virus and the salivary antigens of Ornithodoros spp. ticks, while ticks collected from pig pens were tested for the presence of ASFV.	2006-2007	Quembo et al., 2014
Rwanda				
Senegal	Of 747 serum samples examined, 126 were positive for ASF, suggesting a prevalence of 16.9%.	Seroprevalence survey of ASF in Senegal in 2006, from a sample of pigs in the 3 main pig-farming regions using an ELISA.	2006	Etter et al., 2011
South Africa	Of 98 warthog burrows inspected for Ornithodoros presence, 59 (60.2 %) were found to contain tampans and tick sampling was significantly male biased.	Prospective virus surveys targeted at adult Ornithodoros ticks: a re-evaluation of Mkuze Game reserve, South Africa using PCR based virus detection method, developed specifically for the sylvatic tampan host	2002	Arnot et al, 2009 [28]
Tanzania				

Uganda	Prevalence of ASFV in slaughter pigs was 52.96% (95% CI, 48.75-57.14) and 11.5% (95% CI, 9.06-14.45) by ELISA and PCR respectively. In surveillance districts, the proportion of ASFV positive pigs was 53.59% (95% CI, 46.33-60.71) and 0.55% (95% CI, 0.1-3.06) by ELISA and PCR respectively.	Cross-sectional seroprevalence and virus prevalence of ASFV in apparently healthy slaughter pigs at Wambizi slaughterhouse in Kampala city, Uganda. We also estimated the presence of ASFV antibodies and circulating viral antigens in pigs from selected districts of Uganda during targeted surveillance. Analysis of 540 and 181 blood samples collected from slaughter pigs and pigs from targeted surveillance districts respectively.	2012-2013	Atuhaire et al. 2013, [29]
	The prevalence of ASFV in Rakai was 3.3% while the seroprevalence was 2.1%.	Prospective survey to estimate the prevalence of ASFV and the seroprevalence in domestic pigs in the Rakai district in southern Uganda	2010	Björnheden, 2011
	3.8% (38/997) of the pigs examined had clinical signs and post- mortem lesions suggestive of ASF. Two of 997 (0.2%) sera analysed were positive for ASF antibodies.	A cross-sectional convenient-random sampling strategy was used to examine pigs and collect sera from22 slaughterhouses where individual pigs were randomly selected for a detailed ante- mortem and post-mortem inspections. Sera were also collected for laboratory analysis. A total of 997 pigs (53.7% male and 46.3% female) were examined for lesions suggestive of ASF and seropositivity of sera for ASF antibodies. The sera were tested using enzyme-linked immunosorbent assay (ELISA) and positive	2008-2009	Muwonge et al., 2012

		samples were further confirmed with an immunoblot assay.		
Zambia	The prevalence of infection in 0. moubata was between 0-4% in South Luangwa National Park and 5-1% in Livingstone Game Park	A survey of the distribution of ASF virus in Zambia was carried out by VI from Ornithodoros moubata ticks collected from animal burrows in National Parks and Game Management Areas in northern, eastern, central and southern Zambia. ASF virus was isolated from ticks in all areas examined.	NA	Wilkinson et al., 1988

Conclusions

Since the first recorded cases in the 1940's there has been a general increase in the incidence of ASFV outbreaks in Africa. A 10-year peak period in reporting ASFV is observed between 2002 and 2013 and Rwanda, Malawi and Madagascar accounted for over half of the total number of outbreaks. With the continuing and increasing transmission of ASFV to the Caucasus region of Asia and the Russian Federation, prevention of further incursion into south and Southeast Asia will be critical in the years to come. China, South and Southeast Asia account for over half of the world's pig population in a diverse set of production systems. The introduction of ASFV remains an imposing and enormous risk to animal health and food security globally.

Economic and Social Impacts at Global and Regional Levels, and in Selected Countries

Losses of animals of different species are calculated as Livestock Unit (LSU) losses, using the definitions presented in Table 10 [31]:

Table 10: Definition of species-specific livestock units values based on the World Bank Livestock Disease Atlas

1 camel or "other camelid"	=	1.1 LSU
1 cattle	=	0.9 LSU
1 buffalo	=	0.9 LSU
1 horse or mule (equidae)	=	0.8 LSU
1 pig	=	0.25 LSU
1 sheep	=	0.1 LSU
1 goat	=	0.1 LSU
1 poultry bird (chicken, duck, guinea fowl or goose).	=	0.015 LSU

Although Africa has 25 million pigs, a relatively small share of the global pig population, ASF ranks as the fourth highest cause of economic loss among pig diseases globally [31]. Figure 12 presents a summary of the ranking of pig diseases in terms of Livestock Units (LSU) lost, 2006-2009.

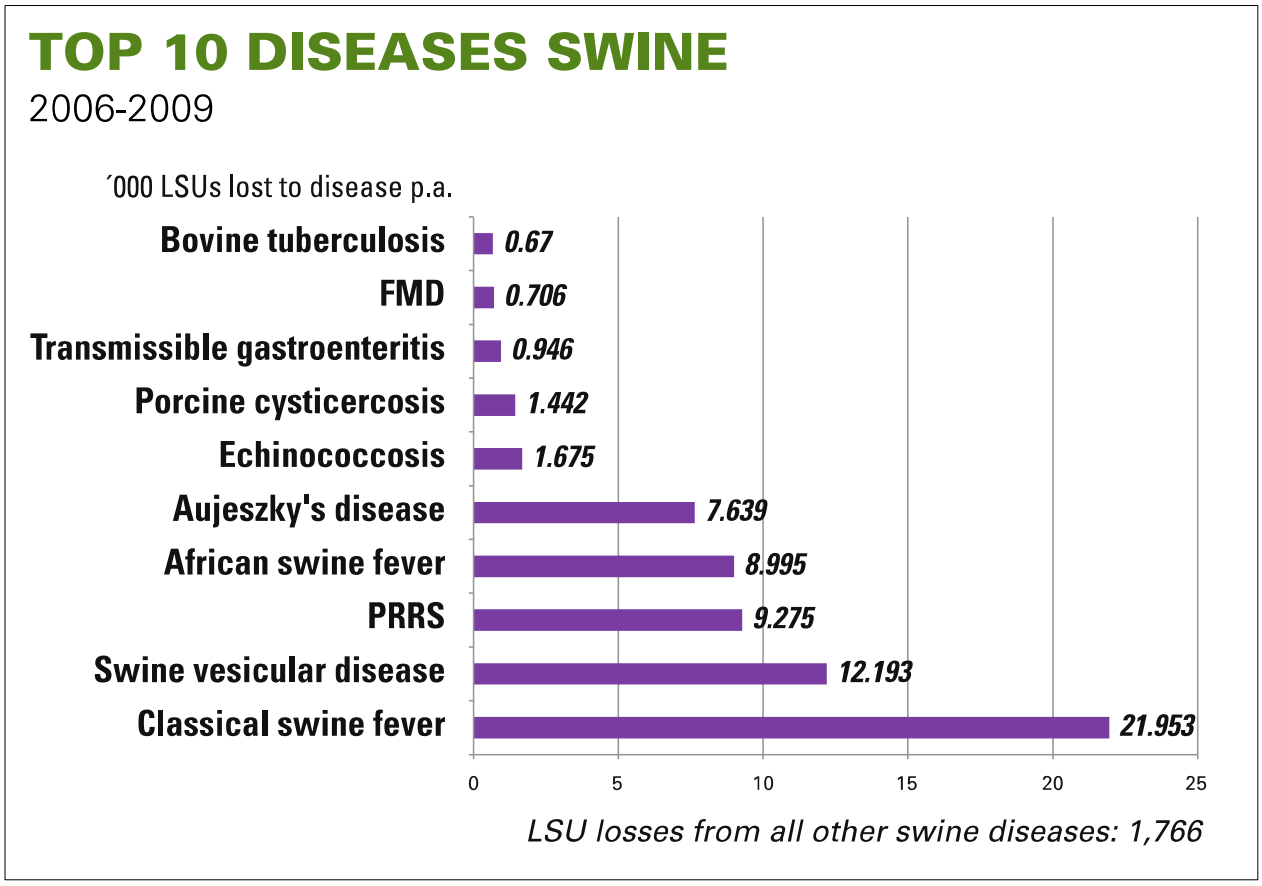

Figure 12: Ranking of the top 10 pig diseases in terms of Livestock Units lost, 2006-2009

Figure 13 summarizes the geographic distribution of the top 10 countries impacted through LSU losses due to ASFV, 2006-2009. In descending order Rwanda, Malawi, Burkina Faso and Mozambique rank among the most affected countries in Africa.

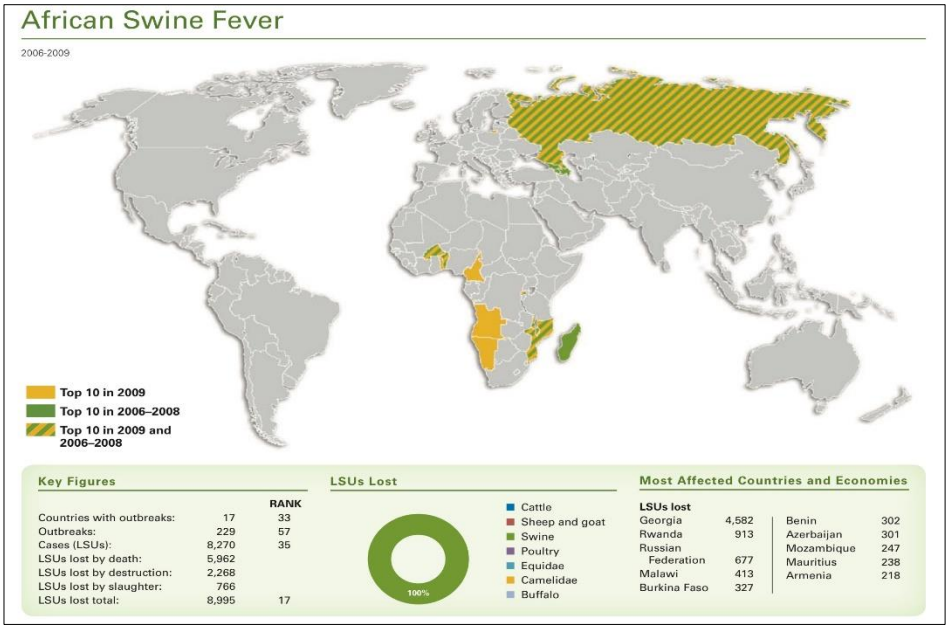

Figure 13: Geographic distribution of the top 10 countries impacted through lost LSU due to ASFV, 2006-2009

Eighty per cent (80%) of pig production in Africa is currently based in smallholder units. However, Figure 14 presents the rapid growth of pig production in Africa based on FAOSTAT estimates for 2000-2010 highlighting the potential future impact of ASFV [20].

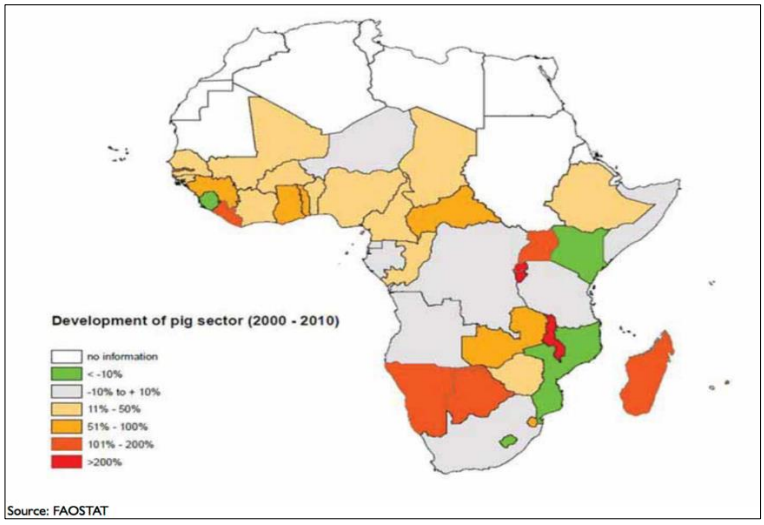

Figure 14: Percentage growth of the pig production sector 2000-2010

One study in the U.S. assessed the cost of maintaining a pig population stricken with ASF over a ten-year period at US$5.4 billion, with the bulk of this being consumer losses [32]. In Spain, the final 5 years of the eradication programme alone were estimated to have cost US$92 million [13]. Estimated economic losses in the Russian Federation in 2009 alone were US$1 billion, with 48,000 pig deaths based on OIE data [33].

Thus far, the greatest impact of ASFV has been on the poorer pig producers lacking basic biosecurity as well as the financial resources to restart production in the absence of compensation schemes [13]. The future cost to intensive pig production systems in Africa will also be significant. Using a model 122-sow piggery unit in West and Central Africa, a financial model and costing were used to estimate the economic benefits of effective biosecurity against ASF. This size of pig production can generate a profit of approximately US$109,637.40 per annum and an outbreak of ASF has the potential to cause losses of up to US$910,836.70 in a single year. The benefit-cost ratio for implementing biosecurity measures is estimated at 29 [34].

Implications of ASFV for food security in Africa are also significant. Pig production is an important source of human dietary protein in many countries, particularly in areas where beef production is difficult. Pigs very efficiently convert food waste and agricultural by-products into high quality protein and they have a relatively short production cycle [13]. Indirect losses are more difficult to estimate and include the loss of international trade and the cost of control measures including culling, government action and loss of production pending release of quarantines. Figure 15 illustrates the trend of live pig sales in smallholder pig units in Rombo district of Kilimanjaro, Tanzania as a result of an ASFV outbreak in 2011 [35].

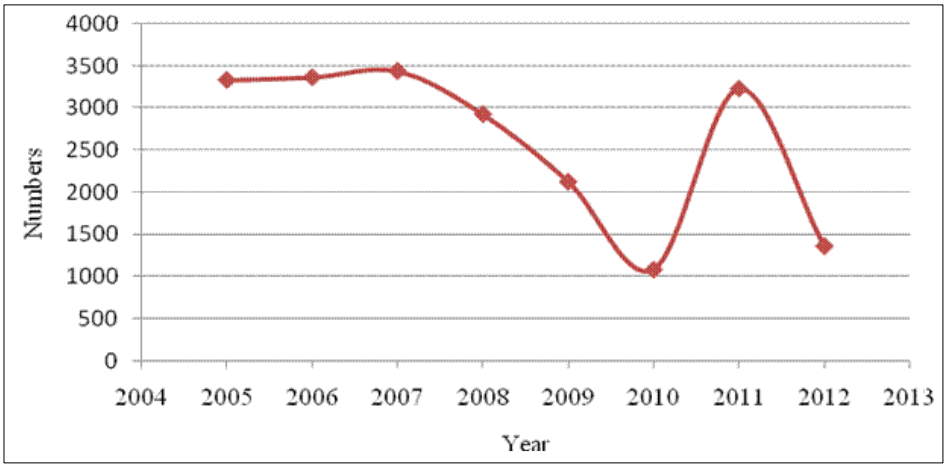

Figure 15: Trend of live pig sales in smallholder pig units in Rombo district of Kilimanjaro, Tanzania

The authors explain that the drop in 2011-2012 is related to ASF outbreaks but do not discuss the reasons for the previous drop from 2007-2010 which appear to be more gradual and may possibly be linked to other value chain dynamics. Table 11 presents a summary the socioeconomic impacts identified for some of the selected African countries where ASFV impact studies have been conducted

Table 11: Prevalence estimates of ASFV in selected African countries

Region/Country	Economic Impact	Social Impact	Year	Reference
Sub Saharan Africa				
Burkina Faso				
Ethiopia				
Ivory Coast	Lack financial resources to restart production in the absence of compensation schemes; Loss of between 30 and 50 per cent of the pig population	The greatest losses are usually inflicted on the poorer pig producers who are less likely to implement effective prevention and control strategies or basic biosecurity.	1988-1989; 2000	Edelsten & Chinombo 1995; El Hicheri et al. 1998; Roger et al. 2001
Kenya				
Madagascar	Loss of between 30 and 50 per cent of the pig population		2000	El Hicheri et al. 1998; Roger et al. 2001
Malawi				
Mali				
Mozambique				
Rwanda				

Senegal				
South Africa				
Tanzania	The impact of an ASF outbreak in smallholder pig units in Rombo district of Kilimanjaro, Tanzania was US$75.7 million based on a study of 1,085 smallholder farmers		2013	Swai and Lyimo, 2014
Uganda				
Zambia	Following the 2011 outbreak in Isoka district of Zambia: 50.0% reduction in pig population in the surveyed area. This ASF outbreak caused 99.9% mortality of affected pigs. The socio-economic impacts of the disease were in terms of loss of the pigs due to mortalities, loss of business and the cost of disease control		2011	Komba et al., 2012

Disease Prevention and Control Methods

Since there is no treatment or vaccine for ASFV, prevention remains the most important means of mitigating the risk of ASFV in pig populations and to reduce the economical losses. FAO has compiled a comprehensive contingency planning manual for ASFV [36].

Treatment (Control)

Medical Treatment

No therapeutic control agent for ASFV currently exists.

Sanitary Control Methods

Since there is no treatment or therapeutic measure available for ASFV, the following interventions have proven to be effective [6][20][36]:

- Rapid disease reporting and zero-reporting at district level;
- Aggressive active surveillance through “case finding”;
- ASFV-recovered carrier swine and persistently infected wild pigs require special consideration in controlling the disease;
- Apply basic components of biosecurity: isolation; movement controls; cleaning and disinfection; and carcass removal;
- Culling affected animals as defined by presumptive (in contact) and confirmed case definitions;
- Early detection through surveillance and strong two-way linking of trained field epidemiologists and laboratory staff;
- Enabling legislation to control through movement restrictions and humane culling;
- Zoning to define the infected zone, the surveillance zone and the free zone;
- Diligent inspection and quarantine services;

- Epidemiological surveillance;
- Humane culling and safe burial;
- Keep affected premises free of pigs and ticks following culling and burial;
- Public awareness campaign;
- Compensation;
- Social support and rehabilitation;
- Verification of freedom from disease.

Prophylaxis (Prevention)

Therapeutic Measures:

An effective vaccine for ASFV is not currently available.

Sanitary Measures:

In order to prevent ASF, the following measures are recommended [6][27][36][37]):

- Free countries:
    - Careful import policy for animals and animal products;
    - Proper disposal of waste food from vehicles, aircraft or ships coming from infected countries;
    - Efficient sterilization of garbage;
- Establish effective border and import quarantine policies and pig production best practices;
- Risk analyses for ASF should provide estimates of the following:
    - Release assessment;
    - Exposure assessment;
    - Consequence assessment;

- Conduct value chain and social network analysis of pig production;
- Epidemiological surveillance based on risk assessments;
- Education to develop informed animal owners;
- Place a ban on swill feeding or implementing controls (cooking) that will make it safe;
- Education and training on improving basic components of biosecurity: isolation; movement controls; cleaning and disinfection; and carcass removal;
- Capacity development and strengthening of laboratories;
- Define pig production compartments to prevent transmission among them.

Options and Strategies for Vaccination

There is currently no safe and effective vaccine for the prevention and control of ASFV. However, the eradication of ASF from Portugal and Spain, after more than 20 years of endemicity, proved that vaccine is not essential in the eradication of this complex disease [4]. The following strategy is recommended for proceeding with the development of an effective vaccine for ASFV based on published literature and consultation with experts:

1. Address the need to conduct basic science to better understand ASFV. Contribute to a long-term, coordinated and systematic analysis of families of antigens and as well as a systematic study of the innate and acquired immune mechanisms of the ASFV. This could be accomplished through facilitating an international network of excellence for antigenic and immune related research.
2. Leverage efforts of research institutions through partnerships with the vaccine industry from the initial stages to develop commercial products.
3. Develop appropriate vaccine models, which can be transferred to private vaccine companies to support a multivalent delivery platform.
4. Develop rapid pen side test for ASFV to support rapid diagnosis that permits differentiating infected from vaccinated animals (DIVA).
5. Develop novel laboratory based cell lines to replace the current reliance on pig macrophage and bone cells, which is cumbersome and can result in variable results.

Government policies related to prevention and control of ASF are presented in Table 12.

Table 12: Official prevention and control policies for ASFV among the 20 selected countries.

ASF	Notifiable	Official surveillance[1] program	Official control [2] program	Vaccination				Treatment/Chemotherapy	
	(yes/no)	(yes/no)	(yes/no)						
Country				Compulsory vaccination	Who pays for the vaccine?	Who delivers the vaccine?	Species vaccinated	Treatment authorised	Frequently practiced
				(yes/no)	(Government, farmers, combination, others-specify)	(official, private vaccinators or both)	(cattle, sheep, goats, pigs, poultry)	(yes/no)	(yes/no)
Burkina Faso									
Ethiopia									
Ivory Coast	Yes	Passif mais actif en cas d'epizootie	Yes	No				No	
Kenya	Yes	Yes, passive	No	N/A	N/A	N/A	N/A	Yes	No
Madagascar									
Malawi	Yes	Yes (passive)	Yes	N/A	N/A	N/A	N/A	N/A	N/A
Mali									

Mozambique									
Rwanda	Yes	Both	Yes	No	No	No	Pigs	No	No
Senegal									
South Africa									
Tanzania	Yes	Yes, passive/active	Yes, in process	N/A	N/A	N/A	N/A	No	No
Uganda	Yes	No	No	(No vaccines)	N/A	N/A	PIGS	Yes (secondary)	Yes
Zambia									
Bangladesh	No	No	No	No	No	No	No	No	No
India									
Nepal	No	No	No	No	N/A	N/A	N/A	N/A	N/A
Indonesia									
Myanmar	No	Yes (passive)	No	No	-	-	-	No	No
Vietnam	No	No	No	No	-	-	-	-	-

[1]Surveillance: is the systematic on going collection, collation and analysis of data and the timely dissemination of information to those who need to know so that action can be taken.
[2]Control: a programme which is approved, and managed or supervised by the Veterinary Authority of a country for the purpose of controlling a vector, pathogen or disease by specific measures applied throughout that country, or within a zone or compartment of that country

Vaccines Available

The first attempt to develop a vaccine took place in 1963 in Portugal but an effective vaccine has not yet been developed. Live-attenuated, inactivated, proteins or recombinant vaccines have been tried unsuccessfully. The reasons for failure include the fact that the ASFV do not induce neutralizing antibodies and the ASFV are highly variable genetically due to homologous reassortment of the MGF [4]. In addition, it has been demonstrated that mainly CD8 T cells of multiple antigenic specificities are activated after ASFV infection in pigs explaining why immunization using only one epitope has failed [4].

The following avenues for vaccine research have been pursued:

- Inactivated Vaccine does not produce any protection based on antibody production and challenge studies [4].
- Recombinant Baculovirus-based Vector, BacMam-sHAPQ expressing a fusion protein as well as ASFV antigens p54, p30 and the extracellular domain of the viral haemagglutinin has been developed. The, BacMam-sHAPQ vaccine induced specific T-cell responses directly after *in vivo* immunization. However, no specific antibody responses were detectable prior to ASFV challenge [38].
- Subunit Vaccine from Spain is currently being developed (Fernando Rodriguez, Centre de Recerca en Sanitat Animal (CReSA), UAB-IRTA, Campus de la UAB, 08193 Bellaterra, Barcelona, Spain.
- Live-attenuated Vaccine (by passages in cultured Vero cells) protects some pigs against challenge with the homologous strain of virus, but the possibility of some of these pigs becoming carriers and developing chronic lesions exists increases when a large number of pigs are vaccinated. Other studies have shown that serum from pigs resistant to homologous and some heterologous strains of ASFV inhibit (in vitro) infection of cells with different, but related, heterologous strains [4].
- Recombinant Vector Vaccines using Aujezsky's disease virus and Adenovirus targeting innate and acquired immune mechanisms (humoral and cell mediated) [4][39].
- Recombinant Proteins of the Same Virus Antigens and Including a Fragment of an Antibody delivery recognizing porcine major histocompatibility complex (MHC) class 2 to pig antigen presenting cells [4].
- Gene Deleted (attenuated) Recombinant vaccine specifically for genes of virulence and immune system evasion or deficient to replicates in cells [4].

The ASFV genome has between 150 and 165 genes. Table 13 provides a summary of some important ASFV candidate genes considered for use in the vaccine models noted above [4][39]:

Table 13: ASFV candidate genes considered for use in the vaccine models

ASF Genes	Role	References
CD2v	Adhesion protein that mediate the haemadsorbtion on red blood cells accelerating virus propagation in pigs, delaying the onset of the disease	Borca et al., 1998
A238L	Modulation of the immune response by interfering with the induction of pro-inflammatory cytokines; inhibiting the NFAT transcription factor controlling the activation of lymphocytes	Tait et al., 2000; Miskin, 1998 and 2000
j4R	A virus–encoded ubiquitin conjugating enzyme having a role in the modulating the host gene transcription pathway	Goatley et al., 2002; Bulimo et al., 2000
genes belonging to the MGF	Virus virulence	Tulman and Rock, 2001
p54 and p72	Prevent virus attachment	[4]
p30	Inhibits virus internalization	[4]
D117 and EP152R	Expressed early in infection and are associated with IL (IL8, IL12, IL1a, IL4) and TNF expression in macrophages	[39]

Products available <u>in 20 selected countries</u> and doses used:

None.

Commercial vaccines manufactured in Africa and Asia

None.

Commercial vaccines imported into Africa and Asia

None.

Characteristics of Ideal Vaccine Candidates for Smallholders

Target product profile

Attribute	Minimum (currently available vaccine)	Ideal
Antigen	Not applicable	Select based on systematic review of all ASFV antigens
Indication for use	Not applicable	Prevent and control ASFV replication and shedding
Recommended species	Not applicable	Domestic Pig
Recommended dose	Not applicable	To be determined – initial: 0.5 ml; booster ideally 1-2 ml
Pharmaceutical form	Not applicable	Options: Inactivated non-replicating recombinant (gene deleted) with DIVA capability or replicating vector based delivery
Route of administration	Not applicable	Intramuscular
Regimen – primary vaccination	Not applicable	To be determined – ideally 45 days of age
Regimen – booster	Not applicable	To be determined – ideally every 6 months
Epidemiological relevance and use for smallholders	Not applicable	Ideally cross-protective across genotypes
Recommended age at first vaccination	Not applicable	With no maternal immunity - Ideally by 45 days of age
Onset of immunity	Not applicable	7-10 days post vaccination

Duration of immunity	Not applicable	Ideally 6 months
Expected efficacy	Not applicable	90-100%
Expected safety	Not applicable	100%
Withdrawal period	Not applicable	Ideally 21-42 days
Special requirements for animals	Not applicable	No possibility of reversion to virulent form or tissue granulomas; multivalent formulation with other priority pig diseases
Special requirements for persons	Not applicable	No tissue granulomas and no possibility of anaphylaxis
Package size	Not applicable	20 doses for smallholders up to 100 doses
Price to end user	Not applicable	Similar to costs for Aujesky's disease*
Storage condition and shelf-life as packages for sale	Not applicable	Keep frozen*
In-use stability	Not applicable	18 months*

* Based on gene deleted vaccine for Aujesky's Disease

Key Conclusions Related to Vaccination

The key challenges to developing a safe and effective vaccine for ASFV include, lack of understanding of the agent-host relationship including:

- Mechanism of immune system evasion for ASFV both innate i.e. macrophages and monocytes and acquired immune systems including both humoral and cell-associated aspects;
- Inability of the pig host to develop neutralizing antibodies to ASFV;
- Differences among the 22 known genotypes;
- Antigenicity of the approximately 150-165 genes of ASFV to target the development of a safe and effective vaccine;
- Dynamics of undergoing internal re-assortment and presence of MGF which impedes the ability to develop an effective and safe vaccine;
- Coordination of basic research among international experts;
- A model for both knowledge generation and technology transfer to vaccine developers for commercial vaccine production;
- Regional- and country-specific vaccination strategies to deal with the unique epidemiological drivers of ASFV of each area.

Limitations

Methodology

There is very limited country level information available concerning the epidemiology and socioeconomic impact of ASFV with the exception of Mozambique and Uganda. Reporting bias presents a significant handicap for estimating country-based risk for A in section 3. However, the OIE incidence country estimates are complemented by AU-IBAR for at least one year. It is evident from a review of the literature that communities in Africa have become accustomed to dealing with ASFV. Community engagement and understanding the knowledge, attitudes and practices (KAP) at the local level in relation to ASFV will therefore a critical consideration for any future vaccination initiative and AusAID is supporting such social science research.

Several international experts were not available or did not respond to requests for an interview.

Gaps in knowledge or capacity impacting strategic planning and effective implementation of a vaccination program for ASFV

A replicating vector vaccine model holds the promise of on going immune stimulation from the vector virus however for wider eventual acceptance a non-replicating, gene deleted vaccine model may be preferred due to lack of environmental concerns and both approaches are being taken internationally at this time. This should be considered as a potential limiting factor.

The development of safe and effective vaccines for ASFV may be 10 years ahead due to the limited amount of basic research, which has been conducted to date. Culling remains an effective means of eradicating ASFV from European countries over prolonged campaigns. Reliance on humane culling used successful in Europe to control ASFV, is not possible due to food security and technical and financial constraints evident in developing countries. Nor is sufficient epidemiological and laboratory capacity available to rapidly detect and diagnose the disease. The key gaps and solutions that need to be addressed prior to developing a potential vaccine are as follows:

Short term

- Facilitating coordination among international researchers to advance knowledge of the basic science of ASFV through a systematic approach for antigenic and immune related research;
- Building local capacity for rapid detection including the development of a rapid pen side test for ASFV to support rapid diagnosis and permits differentiating infected from vaccinated animals (DIVA);
- Conducting epidemiological and socioeconomic studies at the country and transboundary levels to understand the drivers and impacts of ASFV, including introduction, transmission and endemicity;
- Leveraging efforts of research institutions through partnerships with the vaccine industry from the initial stages with potential to develop commercial products.

Medium term

- Study infection dynamics based on direct challenge and indirect contact from infected animals;
- Develop vaccine discovery models based on evidence from antigenic and immunological studies;
- Promoting technology transfer to private vaccine companies to support a multivalent delivery platform.

Long term

- Developing vaccines and vaccine delivery systems appropriate for each country and region based on molecular, epidemiological and socioeconomic parameters.

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